DIGESTION OF LEAF LITTER MATERIALS BY THE SPRINGTAIL TOMOCERUS FLAVESCENS (INSECTA, COLLEMBOLA)

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ПОТРЕБЛЕНИЕ ЛИСТОВОЙ ПОДСТИЛКИ НОГОХВОСТКОЙ TOMOCERUS FLAVESCENS (INSECTA, COLLEMBOLA)

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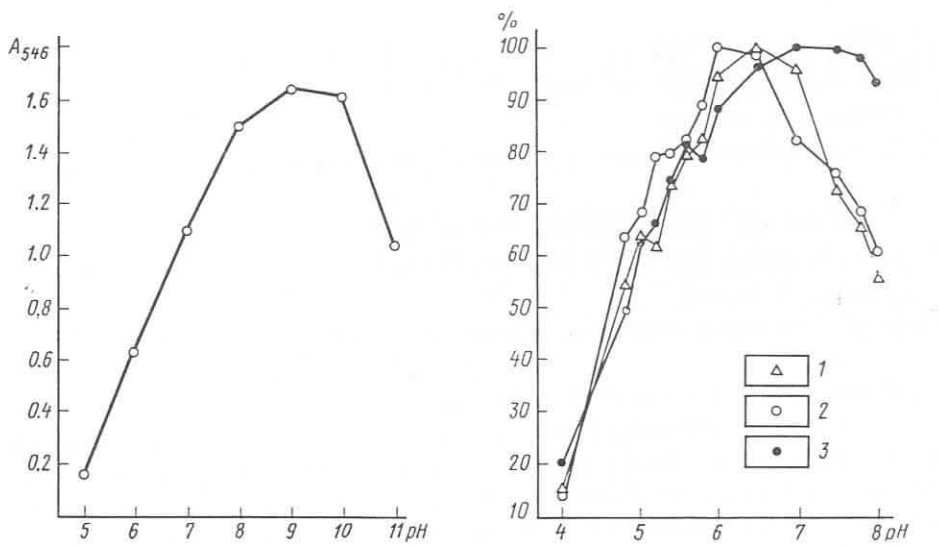
Leaf litter feeding collembola are mainly detritivores. They ingest a complex mixture of plant-cell wall material, fungal mycelia, bacteria and green algae. Although detritus is a potentially rich energy source, this material is generally considered to be of low nutritive value. Furthermore, a good deal of the ingested food seems to pass the alimentary canal only mechanically degraded. Recently has presented careful studies on the feeding biology of Tomocerus flavescens (Tullberg 1871) were presented /2/. Which food components are really hydrolyzed, still remains an unanswered question. We know from our own measurements that the respiratory quotient of T.flavescens is 0.92±0.03 (n=8). Therefore, assimilated food seems to be a mixture of carbohydrates and proteins. Investigations of carbohydrase

and proteinase activities were undertaken in order both to complement the nutritional biology and to clarify digestive capabilities.

The present investigation is an examination of the digestive biochemistry of intact guts of the springtail T.flavescens. Because of its considerable length this species appeared to be most suited for microdissections. Furthermore, T.flavescens is the dominant species of the collembolan population of a beech wood near Bochum University. The midgut of T. flavescens has the shape of a simple tube that occupies nearly the whole dorsal part of the animal. The preparation of a gut from a decapitated animal turned out to be very simple: with two pairs of forceps and some experience it is possible to remove the intact alimentary canal at the abdominal end. The gut was homogenized and the product of this step was centrifugated. Hydrolytic activities of the supernatant (at 25°C) were then identified by microbiochemical tests /4/.

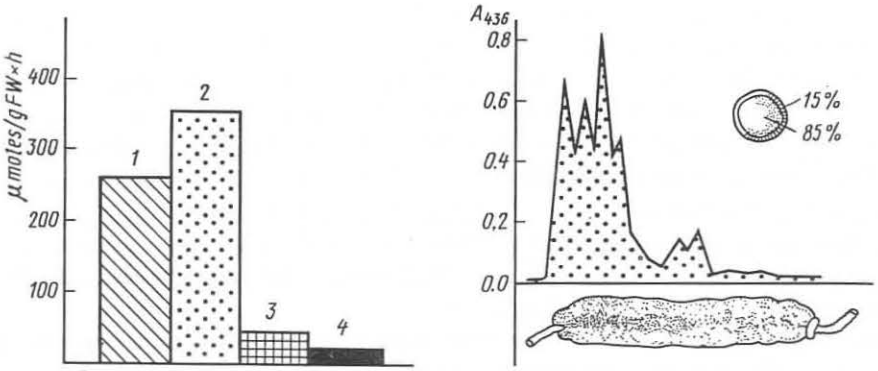
General proteolytic activity was measured by the release of dye from Azocoll (Calbiochem 194931) as substrate. Midgut homogenates posses high activity toward this substrate. Maximum activity occurs at pH 9.0 (Fig.1). Furthermore, gut contents is able to attack the gelatine layer of a fogged film-piece. The black emulsion consisting of silver grains disappears (Fig.1, insert).

The main interest of our experiments was focused on carbohydrase activity. As a first step we estimated the hydrolysis of various disaccharides in dependence of pH. Maximum activity in a midgut extract occurs for sucrose and cellobiose at pH 6.5, for maltose at pH 7.0 (Fig.2). The pH optima are in accordance with the pH values of the collembolan midgut contents.



F i g. 1. Dependence of midgut proteolytic activity (ordinate) on pH. The insert shows the digestion of gelatine layer of a fogged film-piece

F i g. 2. Effect of pH on the hydrolysis of cellobiose (1), sucrose (2) and maltose (3), by midgut extract (n=2)



F i g. 3. Hydrolysis of maltose (1), sucrose (2), trehalose (3) and cellobiose (4) by midgut homogenates (n=15)

F i g. 4. Distribution of sucrase activity within the midgut. Enzyme activity was assayed by incubating cryostat sections (10 µm) of whole animals

As a next step we estimated the activity of disaccharidases quantitatively. The most effective carbohydrases were those that hydrolyze substrates with α-glucosidic linkages such as sucrose and maltose (Fig.3). 85 per cent of the sucrase activity was found in the midgut lumen, only 15 per cent in the midgut tissue. The distribution of carbohydrase activity within the whole midgut is heterogeneously. Hydrolytic activity toward sucrose is highest in the anterior part of the midgut (Fig.4). The activity toward trehalose and cellobiose was comparatively weak, but nevertheless significant. Carefully done control experiments confirmed that the source of the cellobiase activity was not the detrital food. The fact that T.flavescens is provided with a β-glucosidase is of special importance. Its natural function is usually assumed to be associated with the last step of litter cellulose decomposition.

In a following experiment we tested the activity of gut extracts toward a variety of polysaccharide substrates. Only small amounts of glucose (2.7 µmoles/g animal fresh weight per hour) were produced by the hydrolysis of the β-glucosidic linkages of microcrystalline cellulose from cotton linters (Serva 45417). The breakdown of a soluble sodiumcarboxymethylcellulose(Fluka 21900) was not more than twofold. The only substrate toward which the midguts exhibited high activity was starch. Most of the carbohydrase activities were restricted to the alimentary canal, only small amounts of maltase and trehalase were found in the gutless animals.

What are the ecological implications of these patterns of carbohydrases? With regard to the dominance of saccharase, maltase and α-amylase activity in the midgut of T.flavescens the assumption that a preferential hydrolysis only of intracellular compounds of algae, fungal mycelia and bacteria takes place is supported. Slight activity toward carboxymethylcellulose and cellulose indicate that T.flavescens is practically inable of digesting plant cell wall constituents.

To confirm this negative result we fed the animals with ^{14}C-labelled cellulose (New England Nuclear, NEC-732; specific activity 0.034 mCi/mg^{-1}). In order to be successful we had to play a special trick on the animals: the labelled material was blended intimately with unlabelled green algae. Experiments set up to determine the evolution of $^{14}CO_2$ following the consumption of purified ^{14}C-cellulose were carried out in standard Warburg flasks /1/. Three Tomoceri and a distinct meal of Pleurococcus mixed up with 10 μg labelled cellulose were placed in each flask and incubated for 20 hours at 18°C. By that time most of the digested food had passed through the gut. The amount of unconsumed food was minimal.

We were very astonished about the fact that we always recovered a significant number of counts as labelled CO_2 (n=14). 8.7 per cent of the cellulose was metabolized to CO_2. The distribution of radioactivity in other fractions was as following: 9.4 per cent are found in the gutless animal, 4.8 per cent in the alimentary canal and 77.5 per cent in the faeces. The assimilation efficiency of ^{14}C-cellulose was determined to be 18 per cent for a single pulse transit of the gut.

Our findings indicate that our former ecological implications /4/ which base only on enzymatic activity results must be modified. The animals are not only able to digest high amounts of sucrose, maltose and starch. Moreover, they are also able to digest and metabolize certain quantities of β-glucosidic linked substrates. We assume, that intestinal micro-organisms may be responsible for the latter named hydrolysis of substrates that are non-labile residues of plant material. Up to now, we cannot give a satisfying solution of our contradictory results that arise from enzymatic and labelling experiments. One possible interpretation, confirmed by light microscopy, may be the considerable degradation of the purchased labelled substrate in comparison to microcrystalline cellulose fibres. However, degraded cell wall material seems to be more adequate for outdoor conditions.

Acknowledgement

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R e f e r e n c e s

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D i s c u s s i o n

Dallai R.: Have you considered at which intermoult moment animals were sacrified? It may be possible that some enzymatic activity differences exist during this period.

Zinkler D.: Usually, T.flavescens moults every five days and excretes the midgut epithelium after each ecdysis. Animals which were sacrified immediately after field collection sometimes show none or very low carbohydrase activity. Therefore, we only prepared guts of individually kept animals during their feeding and defaecation periods.

Haq M.A.: Do you think collembolans, particularly your species, are responsible for degradation of organic matter; if so, is the enzyme of the animal or with the help of the microorganisms harbouring in the gut of the species studied?

Zinkler D.: T.flavescens especially assimilates "easily soluble compounds of algae" (Wolters 1985). For this purpose the animal produces a wide range of carbohydrases like α-glucosidases, β-glucosidase and α-amylase. In winter, the animal is unspecifically humiphagous. During this time, food material is digested more thoroughly, perhaps with the help of endo- and exoglucanases from micro-organisms.

Petersen H.: Is the duration of the passage of food through the gut sufficiently long to allow bacterial digestion of cellulose and assimilation of the degradation products?

Zinkler D.: Food gut passage of T.flavescens takes 6 to 24 hours. Low temperatures or food shortage may lead to a delayed defaecation. The amount of time should be sufficiently high for an attack by intestinal micro-organisms.